

carboxamidoethylenamine, cholesteryl-3 β -oxysuccinamidoethylenetrimethylammonium iodide, 3 β -(N-(N', N'-dimethylaminoethane)carbamoyl)cholesterol, and 3 β -(N-(polyethylenamine)carbamoyl)cholesterol.

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85. (Once amended) The method of claim 84, wherein said amphipathic adjuvant compound is 3 β -(N-(N', N'-dimethylaminoethane)carbamoyl)cholesterol.

86. (Once amended) The method of claim 84, wherein said amphipathic adjuvant compound is 3 β -(N-(polyethylenamine)carbamoyl)cholesterol.

Remarks

I. Status of the Claims

Claims 25, 30-34, 36-38, 43-47, 49, 55-59, 61, 71-75, 80, 82 and 84-86 have been amended. Claims 25-88 are active in this application.

II. Support for the Amendment

Support for the word "adjuvant" is found throughout the English translation of the specification. For example, support is found in the specification at page 1, line 5; page 2, lines 11-13; page 5, lines 10-12; page 13, line 1; page 14, line 23; page 18, line 15; page 19, line 4; and page 21, line 5. Support is also found in the title and the abstract of the

international application.¹ It is believed that no new matter has been added by this amendment.

III. The Rejections Under 35 U.S.C. § 103 Must Be Withdrawn

Claims 25-86 stand rejected as allegedly obvious over Bolcsak *et al.*, U.S. Patent No. 5,100,662 ("Bolcsak") in view of Gao *et al.*, *Biochem. Biophys. Res. Comm.* 179: 280 (1991) ("Gao"); and separately over (2) Popescu *et al.*, EP 356,339 ("Popescu") in view of Epand *et al.*, U.S. Patent No. 5,283,185 ("Epand"). Applicants respectfully reiterate the traversal of these rejections, and provide the following supplemental remarks.

Applicants have discovered that an amphipathic adjuvant compound possessing a lipophilic group derived from a sterol linked to a cationic group via a carbamoyl group is active as a vaccine adjuvant. The prior art fails to suggest the claimed compositions and methods.

A. The Rejection of the Claims Over Bolcsak In View Of Gao Must Be Withdrawn

Bolcsak provides liposomes and vesicles formed from derivatized sterols. To the extent that Bolcsak teaches a structure that requires a chemical bridge (*see* component (B) in Figure 1 of Bolcsak, at c. 5), Bolcsak fails to suggest that the chemical bridge should be a carbamoyl group.

¹The present application is a continued prosecution application of the U.S. national phase of international application no. PCT/FR95/01495, filed November 14, 1995.

One of ordinary skill in the art would not have been led by Gao to modify the teachings of Bolcsak in an attempt to obtain the claimed invention. Gao relates to a cationic liposome reagent that purportedly facilitates the transfection DNA into cells *in vitro*. Gao is silent concerning an adjuvant effect.

When Applicants' work is contrasted to the prior art, it is clear that lipid adjuvants and transfection agents (taught by Gao) act by different mechanisms. Zhou, X. and L. Huang, *Biochim. Biophys. Acta* 1190: 195-203 (1994) ("Zhou") is of record in the present application as Form PTO-1449 document AR2. In Figure 1, and in the text at page 197 (right column), Zhou teaches that in a complex of DNA and lipopoly(L-lysine) and a co-lipid, substituting DOPC for DOPE as the co-lipid results in severely decreased transfection efficiency.

Zhou's teaching that lipids do not function interchangeably as transfection agents *in vitro* is confirmed by the post-filing art. Attached herewith is the post-filing document Li., S. *et al.*, *J. Controlled Release* 39: 373-381 (1996) ("Li"). Li teaches that substituting DOPC for DOPE the co-lipid results in no transfection efficiency, relative to DOPE. *See* Li at page 377, last paragraph.

In contrast to the teachings in Zhou and Li, in the present application, Applicants show that the adjuvant effect of a combination of DC-Chol and DOPC is equivalent to the adjuvant effect of a combination of DC-Chol and DOPE. *See* pages 9-13 of the English translation of the specification (Examples 6-9).

The fact that DOPC and DOPE can be used interchangeably with DC-Chol to exert an adjuvant effect, but not a transfection effect, means that vaccine adjuvants function by a mechanism that is different than the mechanism by which transfection agents

function. That is true whether an amphiphilic adjuvant compound is used with, or without, a neutral lipid. The difference between adjuvants and transfection agents was not recognized in the prior art. Moreover, the prior art in no way suggests that lipid transfection agents (taught by Gao) function by the same mechanism by which adjuvants function. Therefore, Bolcsak and Gao cannot suggest the claimed invention, and one of ordinary skill in the art would never have combined the teachings of Gao and Bolcsak in an effort to obtain the claimed invention.

B. The Rejection of the Claims Over Popescu In View of Epand Must Be Withdrawn

Popescu provides liposomes formed from dimyristolylphosphatidylcholine (DMPC)/cholesterol liposomes and an antigen. Popescu fails to teach an amphipathic compound possessing a lipophilic group derived from a sterol linked to a cationic group via a carbamoyl group.

Epand purports that a cationic lipid such as cholesteryl-3 β -carboxamidoethylenetrimethylammonium iodide, cholesteryl-3 β -carboxamidoethylene-amine, cholesteryl-3 β -oxysuccinamidoethylene-trimethylammonium iodide, 3 β -(N-(N',N'-dimethylaminoethane)carbamoyl)cholesterol, and 3 β -(N-(polyethylenamine)carbamoyl)cholesterol can be used as a DNA tranfection agent *in vitro*. Epand is silent concerning an adjuvant effect.

Vaccine adjuvants function by a mechanism that is different than the mechanism by which transfection agents function. The difference between adjuvants and transfection agents was not recognized in the prior art. Moreover, the prior art in no way suggests

that lipid transfection agents (taught by Epand) function by the same mechanism by which adjuvants function. Therefore, Popescu and Epand cannot suggest the claimed invention, and one of ordinary skill in the art would never have combined the teachings of Popescu and Epand in an effort to obtain the claimed invention.

C. Summary

Applicants have rebutted the allegations that the claimed invention is obvious over the prior art. Accordingly, Applicants respectfully request that this rejection be reconsidered and withdrawn.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance.

If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the

undersigned at the number provided. Prompt and favorable consideration of this
Amendment and Reply is respectfully requested.

Respectfully submitted,

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